

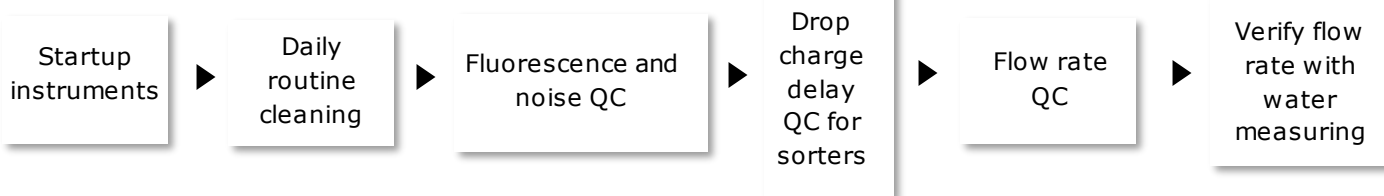


Flow Cytometry  
Core Facility

Ver. # 1.0  
2022-04-04

## Workflow Overview

Description of workflow



## Purpose

Monitoring the stability of the fluid flow is crucial in making sure the instrument is performing correctly. Instability of the fluid flow indicates disturbances in the fluidics system including blockage or pressure issues.

## Supplies Needed

- SH800/MA900 Setup Beads, SONY Biotechnology Inc., Cat # LE-B3001 (Ready to use, do not need to dilute, kept at 4°C, protected from light)
- Falcon Round-Bottom Polystyrene test tubes, Fisher Scientific, Cat #14-959-5
- Falcon Sterile Polyethylene Snap Caps, Fisher Scientific, Cat # 14-959-48
- Balance with readability of at least 0.01g

## 1. Startup instruments

Startup the instruments following their own Startup procedures.

**Note:** Check ► *FACSAria™ Startup, setup and Shutdown.*  
*SONY Startup QC and Shutdown.*

**Note:** Check ► *Analyzer and Diva instructions.*  
*Aurora Startup, QC and Shutdown.*  
*CytoFlex Daily Startup, QC and Shutdown.*

## 2. Daily routine cleaning

Inspect the sheath filter and bleed to remove any air bubbles. Inspect for any particles that may be floating in the filter. If particles are present, sheath filter needs to be replaced. Perform daily routine cleaning for the instruments following their daily cleaning SOPs.

- ✚ Aria: Clean the sample line with CLEAN, RINSE, and WATER at a flow rate of 11 for 2 minutes each.
- ✚ Fortessa: Clean the sample line with CLEAN, RINSE, and WATER at high flow rate for 10 minutes each.
- ✚ CytoFlex: Run system startup program in software with H2O.
- ✚ Aurora: Clean the sample line with CLEAN, RINSE, and WATER at high flow rate for 10 minutes each.

## 3. Fluorescence and noise QC

Perform fluorescence and noise QC for the instruments following their SOPs to ensure that all signals are within expected range. If not, consider cleaning the flow cell.

**Note:** Check ► *FACSAria™ Startup, setup and Shutdown.*  
*SONY Startup QC and Shutdown.*  
*Analyzer and Diva instructions.*  
*Aurora Startup, QC and Shutdown.*  
*CytoFlex Daily Startup, QC and Shutdown.*

## 4. Drop charge delay QC for sorters

Setup Drop charge delay with appropriate QC beads for Aria and SONY following their own SOP.

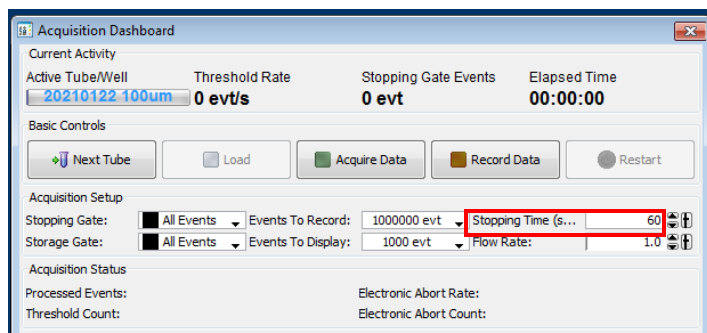
## 5. Flow rate QC with SONY setup beads

Use SONY beads that are in the QC bead box in the refrigerator. If there is no tube with SONY beads or if the tube is empty, prepare a fresh tube of beads by vortexing the vial and then adding 10 drops to a 5ml polystyrene tube. SONY beads are pre-diluted (ready to use), the concentration is about  $5 \times 10^6$  beads/ml. Label the tube with the name of the beads and the date of aliquot, e.g: "SONY SH800 Beads 02/28/21"

### 1. Aria

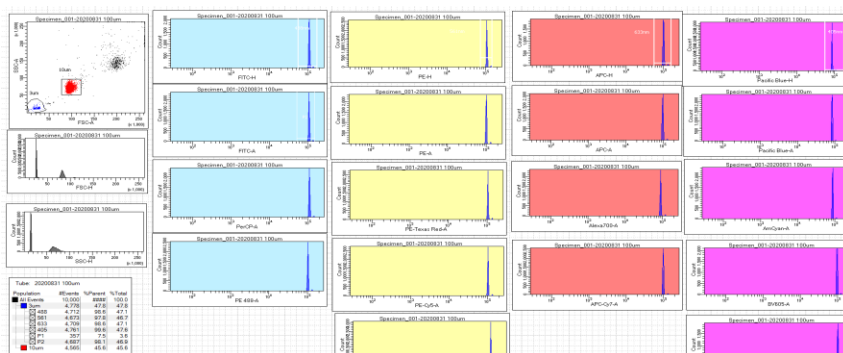
- ✚ Open FLOW RATE Experiment in Diva.
- ✚ Create a new tube in the experiment named as follows: "(year)(month)(date)\_(nozzle size)\_(full tank or half tank)". i.e., 20210228\_100µm\_full
- ✚ Vortex and load SONY beads and run at a flow rate of 1 and wait at least 15 seconds until the threshold rate stabilizes before recording for 1 minute.

- On the Aria, with a full sheath tank, we expect to see 1,000-1,500 events/sec threshold rate with 70  $\mu\text{m}$  nozzle and 2,000-2,500 events/sec threshold rate with 100 $\mu\text{m}$  nozzle. With half a sheath tank, the expected threshold rate will be higher.



| Flow Rate | Flow volume ( $\mu\text{l}/\text{min}$ ) | Expected threshold rate(#/sec) |
|-----------|------------------------------------------|--------------------------------|
| 1         | 10                                       | 833                            |
| 2         | 20                                       | 1,666                          |
| 3         | 30                                       | 2,500                          |
| 4         | 40                                       | 3,333                          |
| 5         | 50                                       | 4,166                          |
| 6         | 60                                       | 5,000                          |
| 7         | 70                                       | 5,833                          |
| 8         | 80                                       | 6,666                          |
| 9         | 90                                       | 7,500                          |
| 10        | 100                                      | 8,333                          |
| 11        | 110                                      | 9,166                          |

Ensure that SONY beads look as expected by scatter and fluorescence. We expect to see one peak fluorescence located around  $1 \times 10^5$  on every channel.



## 2. Fortessa

- Open FLOW RATE Experiment under the QC folder in Diva.
- Create a new tube in the experiment named as follows: "(year)(month)(date)". i.e., 20210228
- Check the Fine adjustment:  
Fortessa1 & Fortessa2 at 500; Fortessa3 at 5 of 10 turns.
- Vortex and load SONY beads and run at low speed. Acquire and wait at least 15 seconds until the threshold rate stabilizes before recording for 1 minute.
- We expect to see 1,500-2,000 events/sec threshold rate at low speed and see one peak fluorescence located around  $1 \times 10^5$  on every channel.



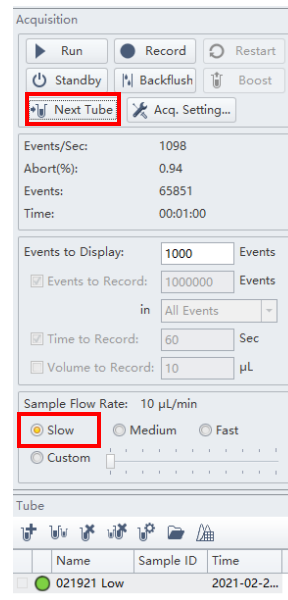
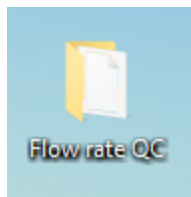
**F1&F2**



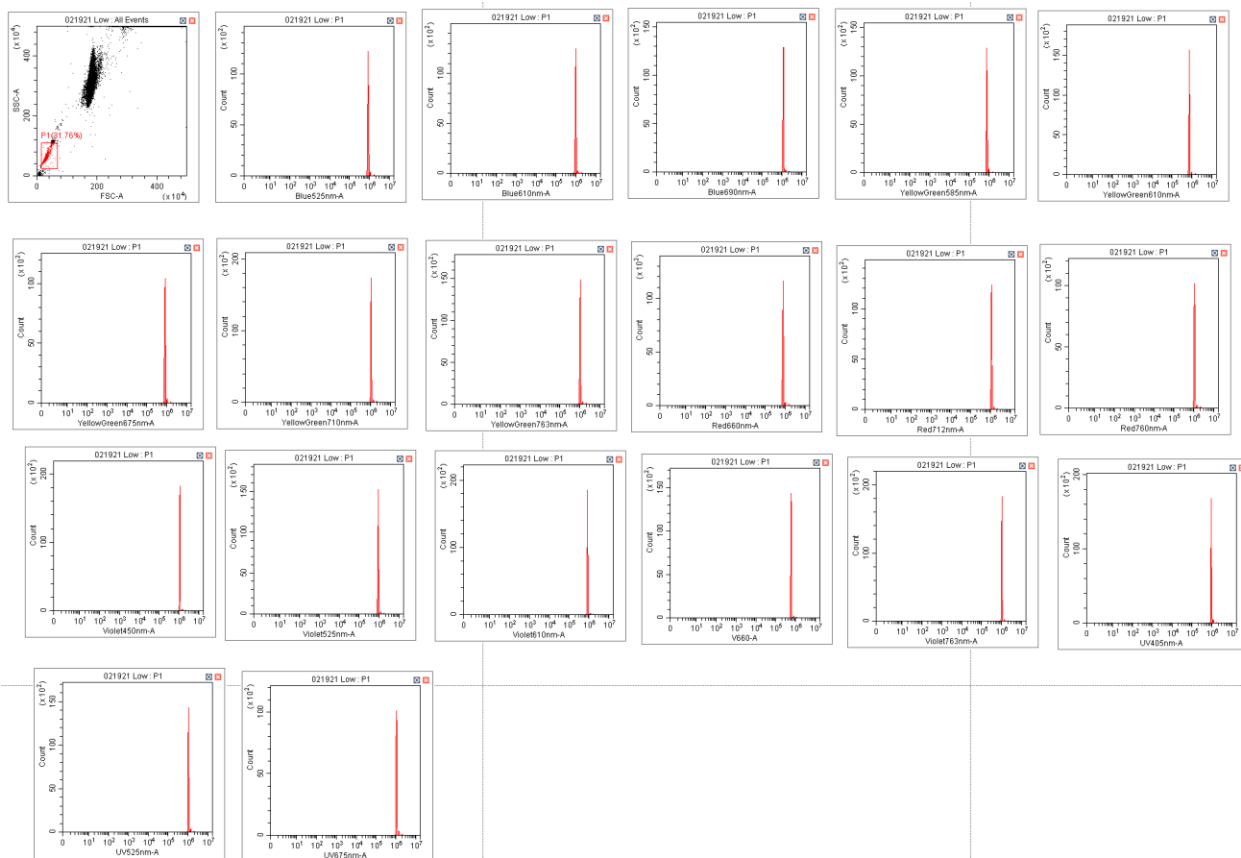
**F3**

### 3. CytoFlex

- ✚ Open FLOW RATE Experiment from Flow rate QC folder on the desktop.

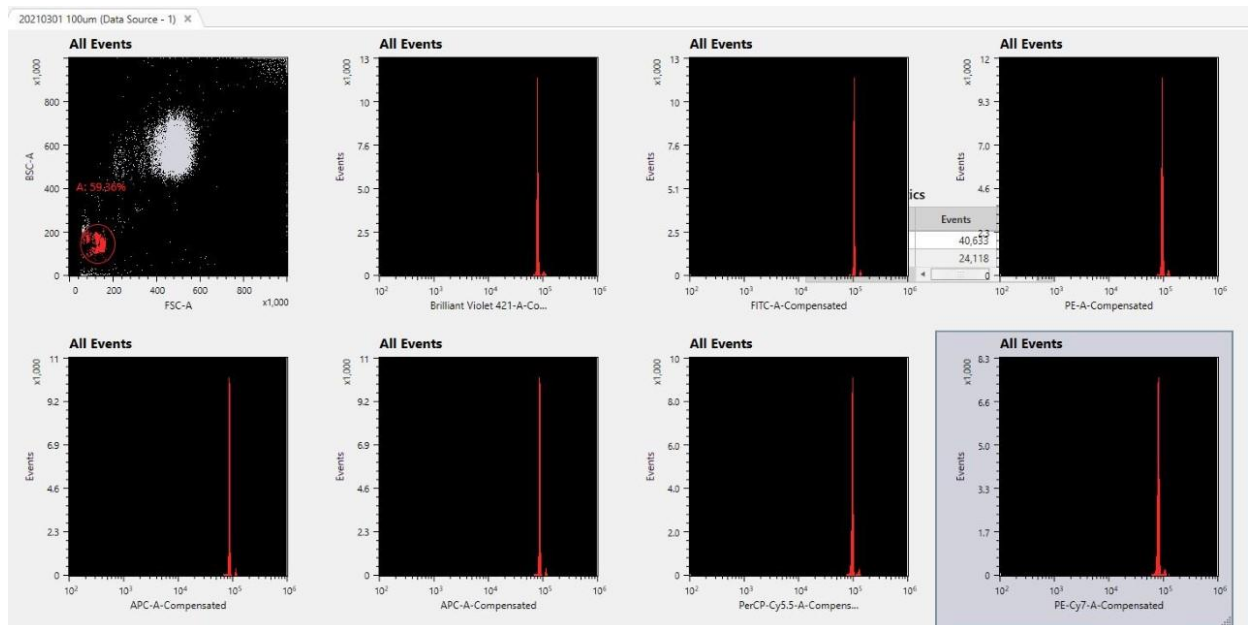
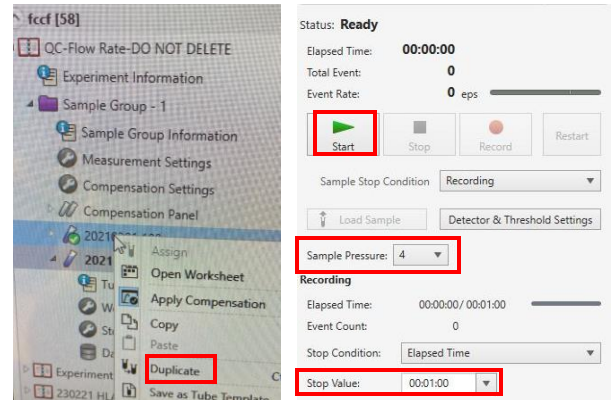
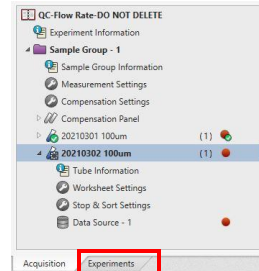


- ✚ Click **Next Tube** to create a new tube in the experiment named as follows: "(year)(month)(date)\_Slow". i.e., 20210228\_Slow
- ✚ Select the flow rate "Slow"
- ✚ Vortex and load the SONY beads. Acquire the beads and wait at least 15 seconds until the threshold rate stabilizes, before recording for 1 minute.
- ✚ We expect to see 1,000-1,500 events/sec threshold rate at low speed and see the peak fluorescence signal located around  $1 \times 10^6$  on every channel.



## 4. SONY

- From the Experiment tab, select the last tube on QC-FLOW RATE Experiment. Right click the tube and click "Duplicate".
- Re-name the new tube as follows:  
"(year)(month)(date)\_(nozzle size)\_(full tank or half tank)". i.e.,  
20210309\_100 $\mu$ m\_full
- Vortex and load the SONY beads and run at sample pressure 4. Go to the Acquisition tab and click **Start**. Wait at least 30 seconds until the threshold rate stabilizes before recording for 1 minute.
- We expect to see 800-1,200 events/sec threshold rate at sample pressure 4 and see one peak fluorescence signal located around  $1 \times 10^5$  on every channel.



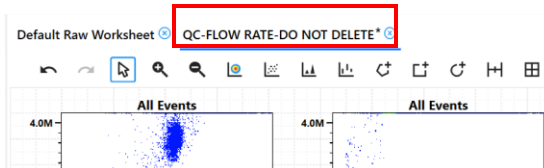
## 5. Aurora

- Open **QC-FLOW RATE** Experiment from **My Experiment**
- Choose **QC-FLOW RATE worksheet**. Add a new tube in the experiment named as follows: "(year)(month)(date)\_Low". i.e., 20210228\_Low
- In the **Instrument Control** panel, select "QC-FLOW RATE-DO NOT DELETE" setting.
- Vortex the SONY beads well. In **Acquisition Control** panel, click **Start** and run beads at **Low** flow rate. Wait at least 15 seconds until the threshold rate stabilizes before recording for 1 minute.
- At **Low** flow rate, we expect to see 1,500-2,000 events/sec for beads and one peak fluorescence signal on every channel. It is located around  $1 \times 10^6$  on 3<sup>rd</sup> channel of each laser.

## My Experiments

Import Export

| Experiment                            | Created By |
|---------------------------------------|------------|
| > 03112021 H82 H526 H446 EdU Wee1 24h | Admin      |
| > QC-FLOW RATE-DO NOT DELETE          | Admin      |



Acquisition Experiment

\*QC-FLOW RATE-DO NOT DELETE

Save Save As Edit Unmix

Tube Group

Group\_001

20210310\_Low

20210312\_Low

Instrument Control

User Settings: QC-FLOW RATE-DO NOT DELETE

Save Save As

GAIN THRESHOLD SIGNAL LASERS

FSC SSC SSC-B

100 15 140

UV Violet Blue YellowGreen Red

UV1 461 UV2 132 UV3 238 UV4 364

UV5 214 UV6 191 UV7 260 UV8 217

UV9 264 UV10 183 UV11 230 UV12 206

UV13 416 UV14 697 UV15 359 UV16 644

All Channels %: 0

Acquisition Control

20210312\_Low : Ready

Start Record Pause Stop Restart

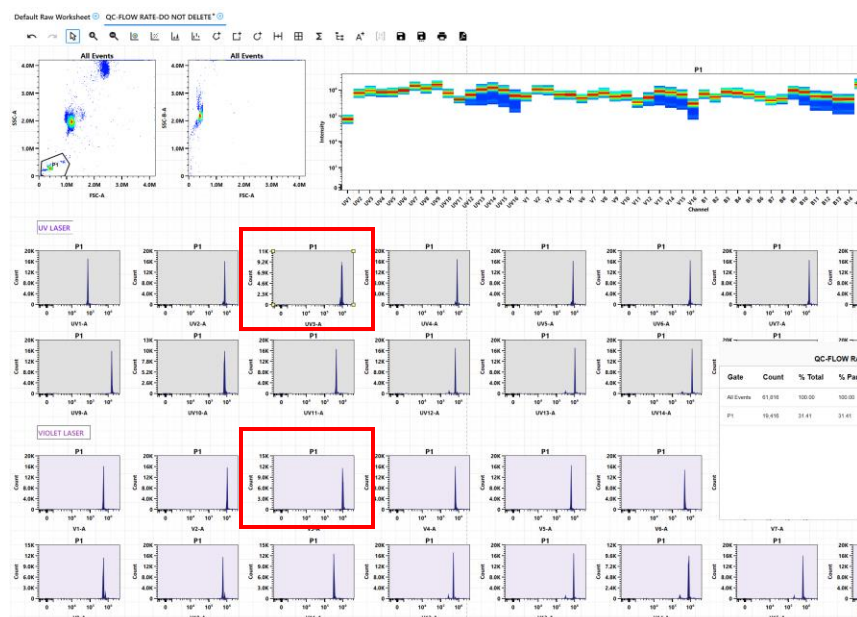
Flow Rate: Low uL/Min: 0.00

Event Rate: 0 Abort Rate: 0

Threshold Count: 0

Time Elapsed: 00:00:00 (HH:MM:SS)

Events to Display: 10,000



## 6. Verify flow rate with water measuring (Optional)

1. Add 3ml Di water to 5ml polystyrene tube and cap it.
2. Turn on the balance.
3. Weigh the tube of water. This weight is A g.
4. Load this tube of water on the instrument and run for 10 mins.
5. Weigh the tube of water afterwards. The weight is B g.
6. Instrument's flow rate( $\mu\text{l}/\text{min}$ )= $((A-B)/10)*1000$



A



B